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# RESIDUES OF DDT, DIELDRIN, AND HEPTACHLOR IN EARTHWORMS DURING TWO YEARS FOLLOWING APPLICATION

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By Charles D. Gish
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# Residues of DDT, Dieldrin, and Heptachlor in Earthworms During Two Years Following Application

by

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Abstract

Study plots were treated at 0.56, 2.24, or 8.97 kg active ingredient per hectare with either DDT, dieldrin, or heptachlor. Earthworms and soils were sampled from each plot when treated and 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 months thereafter, and analyzed by gas chromatography for residues of aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, α-chlordane, γ-chlordane, γ-BHC, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE. Individual and total metabolites in soils and earthworms tended to increase linearly with increasing levels of application. In soils, no significant losses of dieldrin, *p,p'*-DDT, DDD + DDT, DDE + DDD, DDE + DDD + DDT, γ-chlordane, or total heptachlors or chlordanes occurred during the 2 years following application. Heptachlor epoxide and *p,p'*-DDE increased significantly during the 2 years, but heptachlor decreased. In earthworms, maximum residues usually occurred 2–4 months after application and then declined. During the decline, residue levels were cyclic: levels were elevated from late spring to early fall and depressed from late fall to early spring. High levels usually occurred in May and lows in January, coinciding with seasonal high and low activity periods of earthworms. Decomposition of soil organic matter and retention of moisture by earthworms were influenced by levels of application or by chemicals.

Residues of DDT have been reported to accumulate in tissues of earthworms (Barker 1958) and to alter earthworm behavior in removing leaf debris (Baker 1946). Interacting effects of chemical residues in soil, earthworms, and birds have been reported in several studies (Stringer and Pickard 1964; Boykins 1966; Collett and Harrison 1968; Davis 1968; Wheatley and Hardman 1968; Davis and French 1969; Hunt and Sacho 1969; Johnson 1969; Bailey et al. 1970; Dimond et al. 1970; Gish 1970; Korschgen 1970, 1971). However, few reports have contained information on factors affecting the residue relation between soils and earthworms. Because earthworms appear to be generally resistant to organochlorine insecticides (Davey 1963; Edwards 1965), and are eaten by many vertebrate species, an understanding of the relation between residues in earthworms and residues in soils is of value in assessing the dietary effects on vertebrates eating earthworms containing residues.

Study plots were established during 1966 to elucidate some of the factors affecting this residue relation. This report covers data collected between May 1966 and May 1968.

## Materials and Methods

A study area 30.5 × 61 m was established at the Patuxent Wildlife Research Center in December 1963. Rotted barley was mulched over the entire area at about 6,700 kg/ha, and half the area (30.5 × 30.5 m) was later rototilled to a depth of 20–25 cm.

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Although the field containing the study area was periodically mowed, hay within the area was not harvested (except in 1965) and was allowed to remain as ground cover. In May 1966, the study area was divided into 18 plots, 10 m square; aluminum flashing was buried to a depth of 25–30 cm around each plot. The flashing protruded vertically for 30–35 cm before being bent horizontally into each plot, which prevented earthworths from entering or leaving the plots.

The 18 plots were divided between two square blocks, one coinciding with the half of the area that had been mulched and the other with the half that had been mulched and rototilled. All treatments were randomized within each block in a randomized complete block design (Cochran and Cox 1957). The treatments, consisting of wettable powder formulations containing either 50% DDT, 50% dieldrin, or 25% heptachlor, were applied at 0.56, 2.24, and 8.97 kg active ingredient per hectare (ai/ha).

Plots were mowed and raked before any chemicals were applied. Chemicals were mixed with well water and applied by an operator carrying a back-pack spray can while walking at about 1.2 m/s. The solutions were applied under pressure of 2 kg/cm$^2$. The width of the spray boom (1.2 m with three equally spaced nozzles) and the height at which the spray was delivered (30 cm) permitted five parallel passes per plot for each 9.5-L can of solution. Half of the chemical was applied in one direction and the other half applied perpendicular to the first. Each plot was then sprinkled with well water to remove spray from the vegetation and covered by a mulch of barley straw and alfalfa. There was neither precipitation nor noticeable wind during chemical application; temperatures approached 32° C.

### *Sampling Procedures*

Soils and earthworms were collected simultaneously at time of treatment (same day) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 months after application. Samples were collected at random within each plot during each sampling period; however, attempts were made to avoid collection from sites previously sampled. Samples consisted of about 15 g of earthworms and 50 g of soil.

The soil, an Elsinboro loam, is well drained with a 0–2% slope. The top 2.5 cm of soil was sampled with a shovel. Roots, stems, rocks, and soil animals were removed in the field. Soils were placed in acetone-rinsed, wide-mouth quart jars fitted with metal lids and rubber seals; samples were then frozen.

Earthworms were obtained by hand-sorting the soil. Species included immature and adult *Allolobophora chlorotica*, *A. trapezoides*, *A. turgida*, *Eisenia rosea*, and *Lumbricus terrestris*. The most abundant species were *A. trapezoides* and *A. turgida;* least abundant were *A. chlorotica* and *E. rosea*. All species from one plot were pooled into one sample and placed into acetone-rinsed jars while in the field. The jars were placed in a refrigerator for 24 h to allow the worms time to purge soil from the gut. The earthworms were then rinsed lightly with water to remove excess soil, laid on paper toweling to dry slightly, placed into acetone-rinsed, 0.06-L screwtop jars fitted with plastic lids, and frozen.

### *Chemical Methodology*

The wettable powder formulations of technical grade DDT, dieldrin, and heptachlor were analyzed for their active ingredient components. A 1-g sample was weighed into a 100-mL beaker with 5 mL of ethyl ether and 95 mL of hexane. The solution was allowed to stand for 1 week with periodic shakings. Determinations were made with a Jarrell-Ash gas chromatograph equipped with a flame ionization detector. The glass column (4 mm × 2.4 m) was packed with 10% DC-200 on Gas Chrom Q (80/100 mesh). Nitrogen flow was 70–100 mL/min under pressure of 5–10 psi; temperatures were column, 210° C; injector, 210° C; and detector, 250° C. Extraction procedures and residue determinations for earthworms and soils were identical to those reported earlier (Gish 1970).

Residue determinations in the clean extracts were accomplished with a Barber Colman 5360 gas chromatograph equipped with an electron capture (Sr 90) detector. The glass column (4 mm by 1.2 m) was packed with 5% DC-200 on Chromport XXX (70/90 mesh). Nitrogen flow rate was 70–90 mL/min; temperatures were column, 200° C; injector, 230° C; and detector, 240° C. Confirmatory analyses were made by thin-layer chromatography on about 10% of the samples; the results were very similar to those obtained by gas chromatography. For this reason, only gas chromatographic determinations are presented.

Micrograms of lipids in earthworms were determined by evaporating a sample of the extract to dryness. Soil moisture was determined by transferring 2–10 g of soil to a porcelain crucible and placing the crucible into a 100° C vacuum oven for 5 h. The cooled sample was weighed for moisture determination and then placed into a 550° C muffle furnace for 4 h. The cooled sample was reweighed to calculate the organic fraction. Soil pH was determined by adding 50 g of water to 5 g of soil in a beaker which was placed on a magnetic stirrer and read to the nearest 0.1 unit on a Beckman Zeromatic II pH meter.

### *Possible PCB Interference with Insecticide Residues*

Soils and earthworms were collected from each block just before chemical application. Soils averaged 0.006 ppm or less of DDE, DDD, or DDT; worms averaged 0.004 ppm or less of DDE, DDD, DDT, or dieldrin. No other compounds were detected in these

untreated samples. Over 10% of the soil and earthworm samples were checked by thin-layer chromatography; the results were very similar to those from the gas chromatographic analysis. It is therefore unlikely that there was any significant interference from PCB's, either initially or during the study.

### *Recovery Values*

Soil and earthworm samples from a field where pesticides had not been applied were each homogenized and subsampled for analysis. Two subsamples of each were analyzed for organochlorine insecticides. The remaining subsamples were fortified with analytical grade compounds to determine recovery percentages (Table 1). Percent recoveries were corrected for amounts in the untreated samples, but the residue data were not adjusted for the percent recoveries.

### *Residue Values*

Insecticides detected in soils and earthworms are expressed on a parts-per-million, dry weight basis. Because moisture is lost in earthworms held in freezers for extended periods of time (Davis 1966), dry weight seemed to be a more precise method for comparing residues in earthworms with residues in soils.

The limits at which insecticide values could be dependably quantified varied with the sample size. Soil samples usually were of uniform size, and the limits of quantification were either 0.005 or 0.006 ppm, dry weight. At times earthworms were difficult to obtain and quantities varied accordingly. The limits of quantification also varied and were either 0.012, 0.017, or 0.020 ppm, wet weight. Residues detected below these limits are expressed as trace amounts.

### *Statistical Methods*

The variances of the residues were proportional to their arithmetic means. Wheatley and Hardman (1968) made similar observations concerning residues in soils and earthworms. Gish (1970) confirmed these observations, pointing out that residues in soils and earthworms were positively skewed and that logarithmically transformed data presented a more nearly normal frequency distribution. All residue data were transformed to common logarithms before statistical techniques were applied. Trace values were quantified for statistical treatment by dividing the values for the limits of detection by 2 and expressing each quotient on a dry weight basis. The level of significance for differences between values was accepted as $P < 0.05$ unless otherwise indicated. Duncan's multiple range test (Duncan 1955) was used to test for significance in Tables 3, 5, and 6.

## Residue Concentration and Change

### *Dieldrin Plots*

Two subsamples of the wettable powder formulation of "50%" technical dieldrin averaged 44.6% active ingredients. These include 43.1% dieldrin, 1.2% endrin, and 0.3% aldrin.

Dieldrin was the principal residue found in the dieldrin plots, occurring in all samples. Quantities of dieldrin accounted for more than 95% of all organochlorine insecticides in the soil and 93% in the earthworms. Aldrin was detected in 26 of 72 soil samples and in 5 of 72 earthworm samples in amounts at or near the limits of detection. Endrin occurred in 11 of 72 soil samples in amounts that did not exceed 0.2 ppm and in 2 of 72 earthworm samples in amounts that did not exceed 1.0 ppm. The other

Table 1. *Average percentages of insecticides recovered when known amounts were added to soils and earthworms.*[a]

| Compound added | Concentrations (ppm wet weight) of compound added to soils and earthworms | | | | | |
|---|---|---|---|---|---|---|
| | Soils | | Earthworms | | | |
| | 0.05 | 5.0 | 0.5 | 5.0 | 50.0 | 500.0 |
| Dieldrin | 70.0 | 79.8 | 77.8 | — | 79.4 | — |
| *p,p'*-DDE | 109.0 | 107.5 | 96.5 | — | 95.1 | — |
| *p,p'*-DDD | 124.0 | 117.5 | 93.5 | — | 97.2 | — |
| *p,p'*-DDT | 101.0 | 104.2 | 88.3 | — | 91.0 | — |
| *o,p'*-DDT | 107.5 | 111.6 | 95.0 | — | 92.5 | — |
| Heptachlor | 72.0 | 91.8 | 72.5 | 46.0(2) | 94.0 | 31.8(2) |
| Heptachlor epoxide | 81.0 | 92.1 | 79.5 | 92.0(2) | 93.5 | 68.0(2) |
| α-chlordane | 86.0(2) | 90.6(2) | 62.0(2) | 91.0(2) | 60.6(2) | 71.2(2) |
| γ-chlordane | 84.0(2) | 91.4(2) | 58.0(2) | 98.0(2) | 57.5(2) | 73.8(2) |

[a]Each value represents the arithmetic mean of four subsamples, except where indicated by numbers in parentheses. Recovery values were corrected for background levels in soils and earthworms.

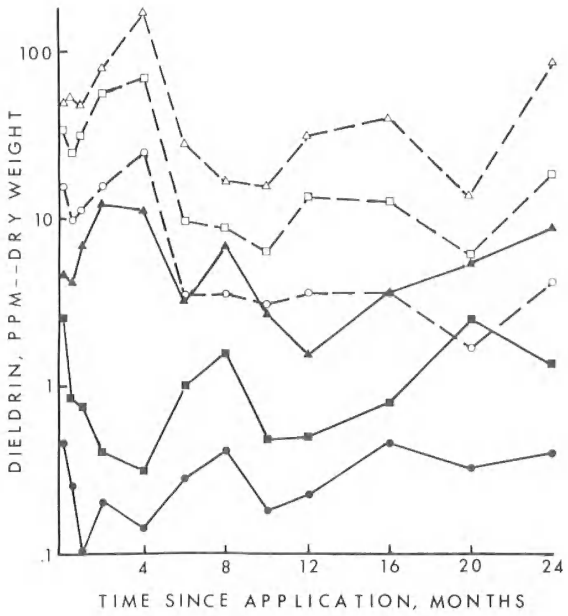


**Fig. 1.** Mean quantities of dieldrin in soils (solid lines) and earthworms (broken lines) at various times following a single application of 50% wettable powder dieldrin at 0.56 (circles), 2.24 (squares), or 8.97 (triangles) kg ai/ha.

organochlorine insecticides detected in these plots included, in decreasing order of abundance, DDE, DDD, *p,p'*-DDT, heptachlor epoxide, and γ-chlordane.

Each line depicting dieldrin levels in soils and earthworms in Fig. 1 represents the arithmetic mean of two replicates (plots); the replicates were not significantly different. Dieldrin in soils and earthworms differed among treatment levels ($P < 0.01$). The relation of dieldrin in soils to the amount applied was parabolic, dieldrin residues increasing at an accelerating rate with increasing levels of application. Dieldrin in earthworms increased linearly ($P < 0.01$) with increasing rates of application.

No discernible loss of dieldrin was detected from soils after 2 years. Infrequently, dieldrin levels differed among sampling periods ($P < 0.01$); however, levels among most periods were not statistically different (Table 2). No distinct trend of increase or loss was evident from the fluctuations of soil residues with time (Table 2) or as tested by simple linear regressions.

A slight decline of dieldrin in earthworms occurred initially, but then dieldrin increased until a maximum was reached in September, 4 months after application (Table 3). At 4 months, earthworms from plots treated at 0.56, 2.24, and 8.97 kg ai/ha averaged 11, 71, and 162 ppm of dieldrin. From a maximum in early fall, there was a sharp decline to a depression in winter through early spring. A subsequent increase to another, less prominent, elevation took place from late spring through early fall, a decrease again in winter, and another increase in late spring. Seasonal fluctuations generally were statistically significant, but subsequent elevations were always less than the maximum at 4 months. Underlying the seasonal fluctuations was a general decline of dieldrin in earthworms during the 2-year study. This decline, illustrated by simple linear regression equations (Table 4), was significant ($P < 0.01$) only for earthworms from the 0.56 and 2.24 kg ai/ha plots.

Dieldrin in earthworms was not significantly correlated with dieldrin in soils over the 2-year period. The low correlation probably was due to the dieldrin fluctuating seasonally in earthworms but not in soils. Correlations were much greater for samples collected within one season, and most were significant.

The ratios of dieldrin in earthworms to dieldrin in soils were also examined. The ratios were calculated by dividing the ppm, dry weight, in earthworms by the ppm, dry weight, in soils, and were called storage–exposure ratios (Table 5). No statistical differences occurred between replications or among dosage levels, but there were differences ($P < 0.01$) among sampling periods. Residues in earthworms averaged 166 times those in soils in the sampling period 4 months after application, when earthworm residues reached a maximum. Fluctuations in these ratios followed the cyclic pattern of dieldrin in earthworms; elevations lasted from late spring to early fall and troughs lasted from winter to early spring. Even during a trough, dieldrin residues in earthworms averaged at least 5.3 times those in soils.

Other variables in this study (Table 6) were examined for their influence on dieldrin concentrations in soils and earthworms by using a stepwise procedure (Draper and Smith 1968) for multiple linear regression. The combined influence of percent organic matter and percent soil moisture, although significant ($P < 0.01$), accounted for only 19.4% of the variability in soil levels of dieldrin. Soil pH, months after treatment, and rainfall during the 2 weeks before sampling did not significantly affect soil levels. About 77.2% ($P < 0.01$) of the variability in earthworm residues was accounted for by the regression equation

$$\hat{Y} = -3.70553 + 0.52248\,X_1 - 0.03787\,X_2 + 0.06699\,X_3 + 0.09025\,X_4 + 0.25352\,X_5$$

where $\hat{Y}$ is the predicted logarithm of ppm dieldrin in earthworms, $X_1$ is the logarithm of ppm in soils, $X_2$ is months after treatment, $X_3$ is the percentage moisture in earthworms, $X_4$ is inches of rainfall during the 2 weeks before sampling, and $X_5$ is the percent lipid in earthworms. This equation describes the relation of dieldrin in earthworms to other measured variables, and may prove useful for predicting quantities of dieldrin in earthworms for some period of years after application.

Dieldrin residues in soils and earthworms were measured in Missouri for 2½ and 5 years (Korschgen 1970, 1971). (Statements of significance concerning these studies

Table 2. *Time response of residues (ppm, dry weight) in soils as illustrated by geometric means.*[a]

| Year and month | Time since application (months) | Dieldrin plots | DDT plots | | | | | Heptachlor plots | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dieldrin | *p,p'*-DDT | *p,p'*-DDE | *o,p'*-DDT | DDD + DDT | DDE + DDD + DDT | Heptachlor | Heptachlor epoxide | γ-chlordane | Heptachlors and chlordanes |
| 1966 | | | | | | | | | | | |
| May | 0.1 | 1.41 ab | 1.11 ab | 0.011 d | 0.18 a | 1.29 a | 1.31 a | 1.30 a | 0.004 f | 0.27 a | 1.61 a |
| June | 0.5 | 0.90 abcd | 0.51 b | 0.009 d | 0.16 a | 0.67 a | 0.68 a | 0.54 ab | 0.055 e | 0.14 ab | 0.77 bc |
| June | 1.0 | 0.68 bcd | 0.49 b | 0.032 c | 0.11 a | 0.61 a | 0.65 a | 0.31 bc | 0.11 de | 0.15 ab | 0.63 bcd |
| July | 2.0 | 0.87 abcd | 0.58 ab | 0.073 b | 0.13 a | 0.71 a | 0.80 a | 0.19 cd | 0.15 cd | 0.11 b | 0.48 cd |
| Sept. | 4.0 | 0.37 d | 0.50 b | 0.076 b | 0.11 a | 0.61 a | 0.63 a | 0.12 cd | 0.24 bcd | 0.11 b | 0.50 cd |
| Nov. | 6.0 | 0.85 abcd | 1.34 a | 0.095 ab | 0.13 a | 0.75 a | 0.86 a | 0.08 de | 0.27 abcd | 0.12 b | 0.54 cd |
| 1967 | | | | | | | | | | | |
| Jan. | 8.0 | 1.64 ab | 0.82 ab | 0.075 b | 0.14 a | 0.96 a | 1.11 a | 0.11 cd | 0.28 abcd | 0.20 ab | 0.62 bcd |
| March | 10.0 | 0.57 cd | 0.74 ab | 0.070 b | 0.17 a | 0.92 a | 0.99 a | 0.09 de | 0.23 bcd | 0.11 b | 0.39 d |
| May | 12.0 | 0.53 cd | 0.50 b | 0.071 b | 0.12 a | 0.62 a | 0.71 a | 0.07 de | 0.24 bcd | 0.11 b | 0.43 cd |
| Sept. | 16.0 | 1.06 abc | 0.61 ab | 0.139 ab | 0.13 a | 0.78 a | 0.94 a | 0.06 de | 0.35 abc | 0.14 ab | 0.61 bcd |
| 1968 | | | | | | | | | | | |
| Jan. | 20.0 | 1.28 abc | 1.09 ab | 0.202 a | 0.18 a | 1.28 a | 1.48 a | 0.07 de | 0.59 ab | 0.27 a | 1.06 ab |
| May | 24.0 | 1.71 a | 0.74 ab | 0.196 a | 0.13 a | 0.88 a | 1.10 a | 0.04 e | 0.68 a | 0.18 ab | 0.98 ab |

[a]Geometric means of three treatment levels, each with two replications. Means that do not share a common letter are significantly different.

Table 3. *Time responses of residues (ppm, dry weight) in earthworms as illustrated by geometric means.*[a]

| Year and month | Time since application (months) | Dieldrin plots | DDT plots | | | | | Heptachlor plots | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dieldrin | *p,p'*-DDT | *p,p'*-DDE | *p,p'*-DDD | DDD + DDT | DDE + DDD + DDT | Heptachlor | Heptachlor epoxide | γ-chlordane | Heptachlors and chlordanes |
| 1966 | | | | | | | | | | | |
| May | 0.1 | 29.6 bc | 1.06 bc | 1.98 cde | 14.7 a | 16.2 a | 18.4 a | 1.26 ab | 4.75 cd | 4.53 ab | 12.0 ab |
| June | 0.5 | 23.0 c | 1.55 bc | 3.30 b | 12.1 ab | 13.6 a | 17.8 a | 1.46 a | 6.00 bc | 3.95 bc | 11.6 ab |
| June | 1.0 | 26.2 bc | 5.23 a | 1.38 def | 9.8 ab | 15.3 a | 16.8 a | 0.39 bc | 2.61 e | 1.58 de | 4.8 de |
| July | 2.0 | 39.9 ab | 2.20 ab | 7.68 a | 10.1 ab | 12.3 a | 23.0 a | 0.25 c | 6.09 bc | 2.64 cd | 9.5 bc |
| Sept. | 4.0 | 50.1 a | 1.15 bc | 2.38 bc | 10.8 ab | 12.0 a | 14.5 a | 0.11 cd | 10.17 a | 6.55 a | 17.2 a |
| Nov. | 6.0 | 9.0 de | 0.59 cd | 1.25 ef | 2.4 de | 3.1 cd | 4.3 de | 0.007 e | 2.42 e | 1.41 e | 3.9 ef |
| 1967 | | | | | | | | | | | |
| Jan. | 8.0 | 7.6 def | 0.24 de | 1.20 f | 2.6 de | 2.8 cd | 4.1 e | 0.004 ef | 2.33 e | 1.49 e | 4.2 def |
| March | 10.0 | 6.6 ef | 0.16 e | 1.79 cdef | 3.5 de | 3.7 cd | 5.5 cde | 0.033 d | 3.23 de | 1.63 de | 4.9 de |
| May | 12.0 | 11.4 d | 0.17 e | 2.14 bcd | 6.7 bc | 6.8 b | 9.1 b | 0.003 ef | 4.45 cd | 1.95 de | 6.5 cd |
| Sept. | 16.0 | 11.7 d | 0.14 e | 2.74 bc | 3.9 cd | 4.6 bc | 7.5 bc | 0.001 f | 4.53 cd | 1.36 e | 5.9 cde |
| 1968 | | | | | | | | | | | |
| Jan. | 20.0 | 5.2 f | 0.15 e | 1.42 def | 2.0 e | 2.1 d | 3.6 e | 0.001 f | 2.10 e | 0.63 f | 2.8 f |
| May | 24.0 | 19.2 c | 0.17 e | 2.42 bc | 4.0 cd | 4.3 bc | 6.8 bcd | 0.001 f | 8.11 ab | 2.64 cd | 10.9 ab |

[a]Geometric means of three treatment levels, each with two replications. Means that do not share a common letter are significantly different.

Table 4. *Simple linear regression equations of residues (Y) from both replicates with time (X).*

| Compounds | Level (kg) | Regression equations[a] Soils | Sig.[b] | Earthworms | Sig.[b] | Correlations: soils with earthworms | Sig.[b] |
|---|---|---|---|---|---|---|---|
| Dieldrin | 0.56 | Y = −0.72694 + 0.01322X | NS | Y = 1.00596 − 0.03052X | ** | −0.287 | NS |
| | 2.24 | Y = −0.22681 + 0.01398X | NS | Y = 1.46456 − 0.02553X | ** | −0.314 | NS |
| | 8.97 | Y = 0.57444 − 0.00043X | NS | Y = 1.68649 − 0.01055X | NS | 0.156 | NS |
| *p,p'*-DDT | 0.56 | Y = −0.64950 − 0.00611X | NS | Y = −0.30350 − 0.04994X | ** | −0.292 | NS |
| | 2.24 | Y = −0.27302 + 0.00711X | NS | Y = −0.02424 − 0.05441X | ** | −0.340 | NS |
| | 8.97 | Y = 0.37482 + 0.01031X | NS | Y = 0.91635 − 0.06600X | ** | −0.299 | NS |
| *p,p'*-DDE | 0.56 | Y = −1.92519 + 0.03072X | ** | Y = −0.12739 − 0.00841X | NS | −0.219 | NS |
| | 2.24 | Y = −1.64113 + 0.05248X | ** | Y = 0.37042 − 0.00161X | NS | 0.080 | NS |
| | 8.97 | Y = −1.16329 + 0.04621X | ** | Y = 0.87287 − 0.00481X | NS | −0.129 | NS |
| *o,p'*-DDT (soils)[c] *p,p'*-DDD (earthworms) | 0.56 | Y = −1.39951 − 0.00500X | NS | Y = 0.50684 − 0.03981X | ** | 0.067 | NS |
| | 2.24 | Y = −0.93849 + 0.00627X | NS | Y = 0.80522 − 0.01795X | * | −0.154 | NS |
| | 8.97 | Y = −0.23921 + 0.00145X | NS | Y = 1.60045 − 0.02118X | ** | −0.064 | NS |
| DDD + DDT | 0.56 | Y = −0.67322 − 0.00194X | NS | Y = 0.57562 − 0.03987X | ** | −0.044 | NS |
| | 2.24 | Y = −0.18589 + 0.00712X | NS | Y = 0.88494 − 0.02109X | ** | −0.239 | NS |
| | 8.97 | Y = 0.47102 + 0.00883X | NS | Y = 1.71160 − 0.02654X | ** | −0.219 | NS |
| DDE + DDD + DDT | 0.56 | Y = −0.62831 + 0.00056X | NS | Y = 0.67898 − 0.03265X | ** | −0.070 | NS |
| | 2.24 | Y = −0.18901 + 0.01237X | NS | Y = 1.03434 − 0.01671X | * | −0.313 | NS |
| | 8.97 | Y = 0.48722 + 0.01005X | NS | Y = 1.78033 − 0.02312X | ** | −0.222 | NS |
| Heptachlor | 0.56 | Y = −1.18115 − 0.04345X | ** | Y = 1.02451 − 0.11172X | ** | 0.667 | ** |
| | 2.24 | Y = −0.52267 − 0.04832X | ** | Y = −0.41837 − 0.14330X | ** | 0.748 | ** |
| | 8.97 | Y = 0.29725 − 0.04592X | ** | Y = 0.09885 − 0.16266X | ** | 0.680 | ** |
| Heptachlor epoxide | 0.56 | Y = −1.49672 + 0.03365X | ** | Y = 0.30742 − 0.01430X | NS | −0.374 | NS |
| | 2.24 | Y = −1.13541 + 0.04624X | ** | Y = 0.71862 − 0.00631X | NS | −0.209 | NS |
| | 8.97 | Y = −1.03703 + 0.07664X | ** | Y = 0.87777 + 0.01614X | NS | 0.525 | ** |
| γ-chlordane | 0.56 | Y = −1.39319 + 0.00300X | NS | Y = 0.14264 − 0.03691X | ** | 0.107 | NS |
| | 2.24 | Y = −0.81212 + 0.00016X | NS | Y = 0.43060 − 0.01411X | NS | −0.202 | NS |
| | 8.97 | Y = −0.37610 + 0.01016X | NS | Y = 0.85965 − 0.00282X | ** | 0.053 | NS |
| Heptachlors and chlordanes | 0.56 | Y = −0.74110 − 0.00120X | NS | Y = 0.57264 − 0.02263X | * | 0.277 | NS |
| | 2.24 | Y = −0.24065 + 0.00424X | NS | Y = 0.95324 − 0.01179X | NS | 0.128 | NS |
| | 8.97 | Y = 0.37337 + 0.00446X | NS | Y = 1.22772 + 0.00513X | NS | 0.008 | NS |

[a]Regression equations are of the form Log Y = a + bX:Y = estimated logarithm of residue, and the antilogarithm must be taken to convert to the geometric mean, ppm, dry weight; a = constant term; b = regression coefficient; X = time in months after single application.

[b]Significant at 5% (*), 1% (**), or not significant (NS).

[c]Only *o,p'*-DDT was found in soil samples and only *p,p'*-DDD was found in earthworm samples.

[Korschgen 1970, 1971] are based on statistical analysis of raw data kindly supplied by Mr. Korschgen). No significant loss of dieldrin from soils occurred in either study or in a 41-month study by Caro and Taylor (1971); these results were confirmed in our study. Only in the 2½-year study (Korschgen 1970) did dieldrin in earthworms change with time ($P < 0.01$) or exhibit an elevation-trough effect.

Dieldrin quantities in earthworms were larger in early June samples than in April and October samples; earthworm residues in April and October in the 5-year study exhibited no elevation-trough effect (Korschgen 1971). Based on our findings, dieldrin in earthworms should increase in April to a late spring–early fall peak and decrease in October to a winter–early spring trough and would be expected to be similar. However, dieldrin in earthworms in both studies (Korschgen 1970, 1971) varied sufficiently that correlations with dieldrin in soils were not significant, similar to our findings. Ratios of residues of dieldrin in earthworms to residues in soils were largest in June of the 2½-year study (Korschgen 1970) and differed very little in the remaining sampling periods of either

Table 5. *Ratio of storage in earthworms to exposure from soils at various times since application.*[a]

| Year and month | Time since application (months) | Dieldrin plots | DDT plots | | | | Heptachlor plots | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Dieldrin | *p,p'*-DDT | *p,p'*-DDE | DDD + DDT | DDE + DDD + DDT | Heptachlor | Heptachlor epoxide | γ-chlordane | Heptachlors and chlordanes |
| 1966 | | | | | | | | | | |
| May | 0.1 | 34.3 c | 2.2 b | 248.8 b | 17.5 ab | 19.7 ab | 1.7 bc | 1,879.0 a | 21.1 bc | 12.5 bcde |
| June | 0.5 | 30.9 c | 5.0 ab | 401.2 a | 24.5 ab | 29.7 ab | 4.3 a | 132.1 b | 31.5 b | 18.8 bc |
| June | 1.0 | 63.5 bc | 12.3 a | 65.7 c | 26.9 ab | 28.0 ab | 2.1 b | 26.6 b | 15.0 bc | 11.7 bcde |
| July | 2.0 | 100.7 b | 7.5 ab | 120.5 bc | 43.3 a | 47.2 a | 1.7 bc | 43.2 b | 28.6 b | 21.3 b |
| Sept. | 4.0 | 166.4 a | 3.7 b | 44.0 c | 27.5 ab | 31.2 ab | 1.7 bc | 46.7 b | 75.6 a | 38.0 a |
| Nov. | 6.0 | 11.2 c | 1.1 b | 14.9 c | 4.5 b | 5.5 b | 0.15 cd | 10.2 b | 16.9 bc | 8.9 cde |
| 1967 | | | | | | | | | | |
| Jan. | 8.0 | 5.5 c | 0.32 b | 18.1 c | 3.1 b | 4.0 b | 0.03 d | 9.6 b | 11.1 bc | 8.4 de |
| March | 10.0 | 13.9 c | 0.22 b | 28.5 c | 4.3 b | 5.9 b | 1.1 bcd | 18.5 b | 18.0 bc | 16.1 bcd |
| May | 12.0 | 22.8 c | 0.41 b | 30.9 c | 12.3 ab | 14.4 b | 0.0 d | 20.7 b | 21.3 bc | 16.3 bcd |
| Sept. | 16.0 | 11.7 c | 5.5 ab | 21.6 c | 8.3 b | 10.5 b | 0.0 d | 16.8 b | 10.0 bc | 10.2 cde |
| 1968 | | | | | | | | | | |
| Jan. | 20.0 | 5.3 c | 0.16 b | 10.5 c | 1.8 b | 2.6 b | 0.0 d | 4.1 b | 2.6 c | 2.8 e |
| May | 24.0 | 11.5 c | 0.28 b | 12.7 c | 5.3 b | 6.4 b | 0.0 d | 13.6 b | 19.1 bc | 12.7 bcde |

[a]Storage:Exposure ratio is calculated by dividing the ppm (dry weight) in earthworms by the ppm (dry weight) in soils. Means that do not share a common letter are significantly different.

study (Korschgen 1970, 1971). Our maximum ratios also corresponded to those periods of peak earthworm residues.

In an Illinois study (Luckmann and Decker 1960), dieldrin levels in soils and earthworms from fields treated from 1 to 5 years before sampling decreased with time. However, no seasonal influences could be evaluated due to the sampling scheme.

### *DDT Plots*

Two subsamples of the wettable powder formulation of "50%" technical DDT averaged 43.2% active ingredients: 35.2% *p,p'*-DDT, 7.5% *o,p'*-DDT, 0.4% *o,p'*-DDD, 0.1% *o,p'*-DDE, and no *p,p'*-DDD, or *p,p'*-DDE. The principal residues in the plots were *p,p'*-DDE, *p,p'*-DDD,

Table 6. *Time relation between environmental factors and residues in soil and earthworms.*[a]

| Year and month | Time since application (months) | Rainfall 2 weeks before sampling (inches) | Mean amounts in soils | | | Mean amounts in earthworms | |
|---|---|---|---|---|---|---|---|
| | | | Percent moisture | Percent organic matter | pH | Percent moisture | Percent lipids |
| 1966 | | | | | | | |
| May | 0.1 | 0.00 | 17.67 cd | 3.50 cd | 6.71 e | 71.24 de | 0.431 h |
| June | 0.5 | 0.41 | 17.17 cd | 3.63 cd | 7.10 c | 71.99 d | 1.019 bc |
| June | 1.0 | 0.73 | 13.69 e | 3.16 d | 6.78 de | 70.82 de | 1.126 b |
| July | 2.0 | 0.20 | 8.08 g | 3.91 abcd | 6.89 d | 78.67 a | 0.806 e |
| Sept. | 4.0 | 7.00 | 17.94 c | 3.82 abcd | 6.72 e | 74.13 c | 0.605 fg |
| Nov. | 6.0 | 0.47 | 16.97 cd | 3.81 abcd | 6.78 de | 71.06 de | 0.529 gh |
| 1967 | | | | | | | |
| Jan. | 8.0 | 0.41 | 19.78 b | 4.59 a | 7.23 bc | 70.89 de | 0.666 f |
| March | 10.0 | 1.28 | 20.49 b | 3.24 d | 7.09 c | 70.53 e | 0.958 cd |
| May | 12.0 | 1.25 | 15.87 d | 3.64 bcd | 6.91 d | 71.88 d | 0.936 cde |
| Sept. | 16.0 | 0.20 | 11.61 f | 4.56 a | 7.41 a | 75.63 b | 0.859 de |
| 1968 | | | | | | | |
| Jan. | 20.0 | 2.26 | 27.03 a | 4.15 abc | 7.30 ab | 73.42 c | 0.846 de |
| May | 24.0 | 1.75 | 20.85 b | 4.47 ab | 7.11 c | 76.28 b | 1.308 a |
| Average | – | – | 17.26 | 3.87 | 7.00 | 73.05 | 0.841 |

[a]Means that do not share a common letter are significantly different.

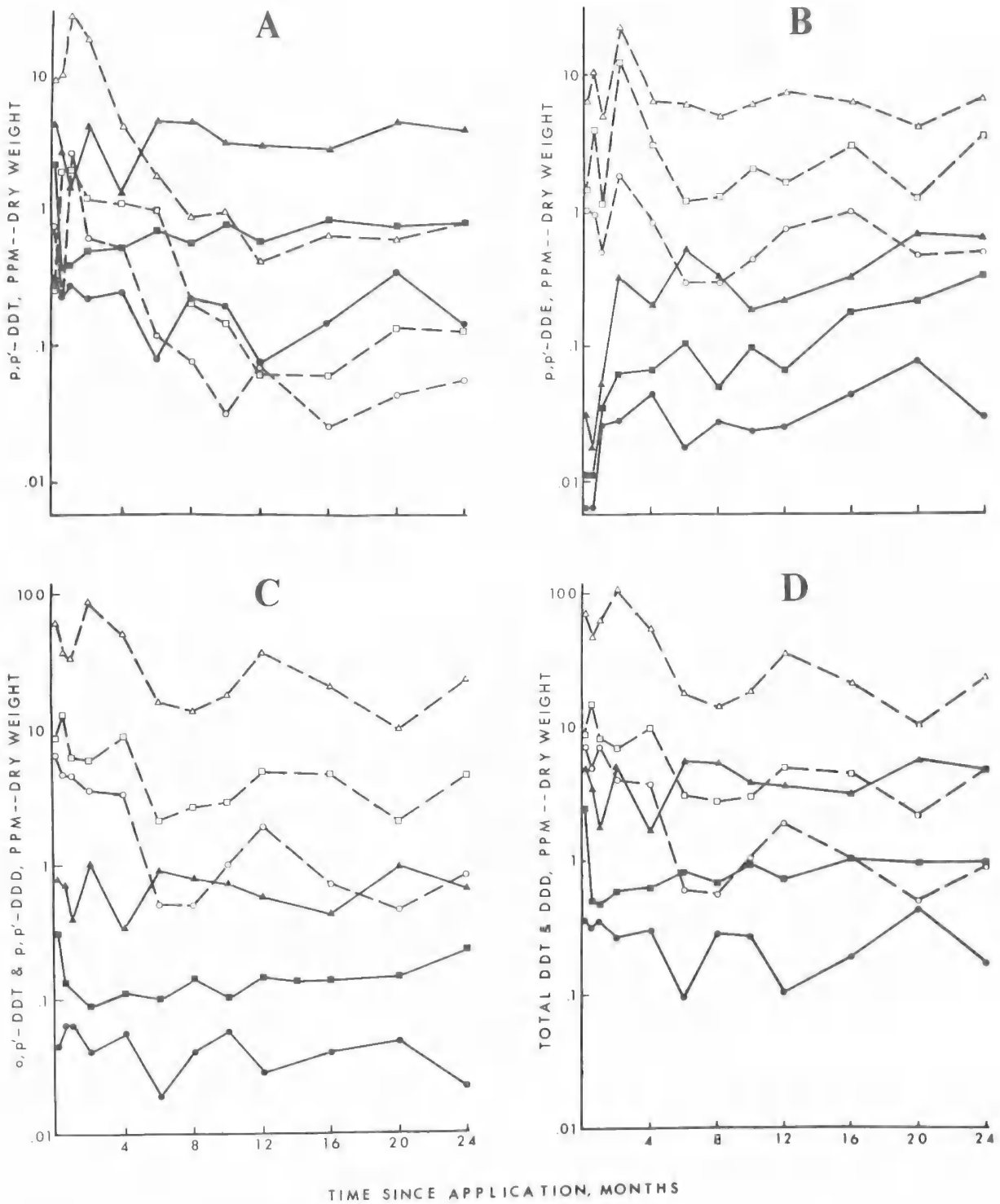


Fig. 2. Mean quantities of *p,p'*-DDT (A), *p,p'*-DDE (B), *o,p'*-DDT (soils only) and *p,p'*-DDD (earthworms only) (C), and DDT + DDD (D) in soils (solid lines) and earthworms (broken lines) at various times following a single application of 50% wettable powder DDT at 0.56 (circles), 2.24 (squares), or 8.97 (triangles) kg ai/ha.

*p,p'*-DDT, and *o,p'*-DDT. These residues made up more than 94% of all organochlorine insecticides present in the soil and 97% in the earthworms. The remaining percentages were composed of dieldrin, heptachlor expoxide, and γ-chlordane. Small amounts of γ-BHC were detected early in the study; no *o,p'*-DDE or *o,p'*-DDD were detected in the samples.

Levels of DDT compounds in soils and earthworms are presented in Figs. 2 and 3, each line representing the arithmetic mean of two replicates. There were no significant differences between replications for any of the compounds in either soils or earthworms. Differences among treatment levels were significant ($P < 0.01$) for all compounds in soils and earthworms. All DDT compounds in soils and *p,p'*-DDT and *p,p'*-DDE in earthworms increased linearly ($P < 0.01$) with increasing levels of application. The relation of *p,p'*-DDD in earthworms to amounts of DDT applied was parabolic, as were the relations of the DDT totals with and without DDE; all residues increased at an accelerating rate with increasing levels of application. Parabolic trends also were present for *p,p'*-DDT and *p,p'*-DDE in earthworms but they were not significant. It would appear that earthworms accumulated quantities of DDT and metabolites at a rate that was geometrically related to the levels of application.

Amounts of *p,p'*-DDE in soils were at a minimum in the early sampling periods and gradually increased to a maximum at 2 years (Table 2 and Fig. 2B); the increase was linear with time ($P < 0.01$) for each treatment level (Table 4). No distinct time trend of increase or loss of the other components or their totals was evident from the fluctuations of soil residues (Table 2) or from the simple linear regressions (Table 4).

Differences among sampling periods were significant ($P < 0.01$) for all DDT compounds in earthworms (Table 3). Quantities of *p,p'*-DDT reached respective maxima of 2.6, 2.0, and 28.0 ppm for 0.56, 2.24, and 8.97 kg ai/ha plots by 1 month after application and decreased to a minimum by 10 months. No significant fluctuations occurred during the remainder of the 2 years. Maximum *p,p'*-DDE values in earthworms of 1.8, 12.5, and 23.0 ppm from 0.56, 2.24, and 8.97 kg ai/ha plots were reached 2 months after application; *p,p'*-DDE then varied from a low in January to a high in September to another low in January and to a high in May. The subsequent elevations were greater than the troughs but less than the maximum achieved 2 months after application. Maximum *p,p'*-DDD levels of 6.4 ppm from 0.56 kg ai/ha plots occurred in the initial sampling, 8.8 ppm from 2.24-kg plots at 4 months, and 89.0 ppm from 8.97-kg plots at 2 months. However, no significant decline occurred until after 4 months when a pattern of elevations and troughs was evident with lows in January and highs in May. Generally, the highs and lows were different from each other and less than the maximum. The patterns for both DDT totals (with and without DDE) were similar to that for *p,p'*-DDD, except that all maximums for DDE + DDD + DDT occurred 2 months after sampling when values averaged 5.8, 19.5, and 131.0 ppm from 0.56, 2.24, and 8.97 kg ai/ha plots.

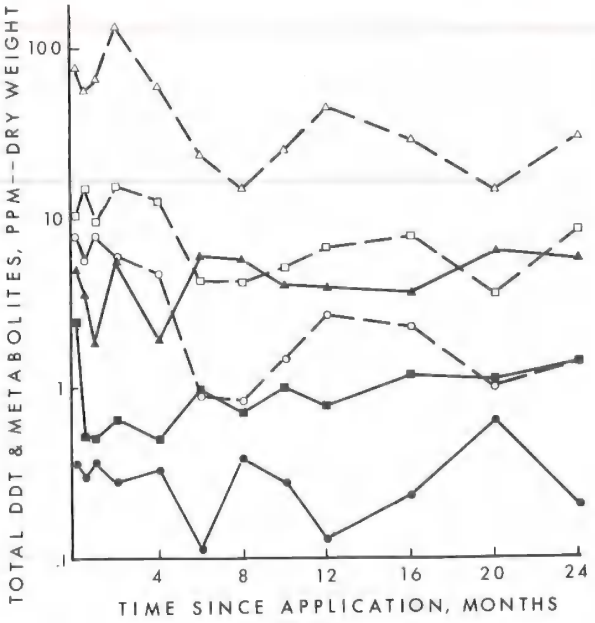


Fig. 3. Mean quantities of total DDT and metabolites in soils (solid lines) and earthworms (broken lines) at various times following a single application of 50% wettable powder DDT at 0.56 (circles), 2.24 (squares), or 8.97 (triangles) kg ai/ha.

All residues in earthworms declined during the 2-year study. The decline of all compounds except *p,p'*-DDE was linear with time ($P < 0.01$) for all treatment levels (Table 4). The levels of *p,p'*-DDE in earthworms after 2 years were less than the maximum found 2 months after DDT application (Table 3) but were not significantly linear with time (Table 4).

Linear correlations over the 2-year period between DDT metabolites (or their totals) in earthworms and quantities in soils were not significant. The low level of correlation probably was due, as with dieldrin, to the residues fluctuating seasonally in earthworms but not in soils. Correlations were much greater, as with dieldrin, for samples collected within one season, and most were significant.

Ratios of residues in earthworms to residues in soils, expressed as ppm dry weight in earthworms divided by ppm dry weight in soils, were examined for differences (Table 5). Generally, the ratios were significant only when the residue levels in earthworms reached a maximum. When *p,p'*-DDT in earthworms was at a maximum, the compound was 12.3 times that in the soil and was significantly greater than at other times, but after 8 months, *p,p'*-DDT was generally higher in soils than in earthworms. After 2 weeks, *p,p'*-DDE in earthworms was

400 times that in soil, but declined thereafter with small, nonsignificant fluctuations corresponding to the seasonal pattern in earthworms. Total DDT metabolites in earthworms averaged 47 times that in soils 2 months after application; ratios fluctuated thereafter, generally following the seasonal pattern. Fluctuations of the ratios with different sampling periods probably explain the lack of significant correlations between soil and earthworm residues for the 2-year period.

Total DDT and metabolites in soils and earthworms were compared by stepwise regression with the other factors measured in this study (Table 6). Percent organic matter and amount of rain during the 2 weeks before sampling influenced soil residues of total DDT compounds, but their influence was not significant. Other factors had no measurable influence on soil residues. However, the other factors were responsible for some of the variability of earthworm residues. Nearly 74.5% ($P < 0.01$) of the variability of total DDT residues could be accounted for by the equation

$$\hat{Y} = -2.47967 + 0.79089\,X_1 - 0.04027\,X_2 + 0.04837\,X_3 + 0.37466\,X_4$$

where $\hat{Y}$ is the predicted logarithm of ppm in earthworms, $X_1$ is the logarithm of ppm in soils, $X_2$ is months after treatment, $X_3$ is the percent moisture in earthworms, and $X_4$ is the percent lipid in earthworms. This equation is merely descriptive, and its limitations have not been determined. It may prove useful for predicting quantities of total DDT and metabolites in earthworms.

The occurrence of gradually increasing quantities of *p,p'*-DDE in soils over the 2 years is consistent with laboratory studies of aerobic soils. The DDT was biodegraded to DDD under anaerobic conditions (Guenzi and Beard 1967, 1968) and disappeared much faster in submerged soil than in aerobic soil (Ko and Lockwood 1968). The main metabolite of DDT in soils under anaerobic conditions was DDD, whereas DDE was the most abundant metabolite under aerobic conditions (Guenzi and Beard 1968). We detected a relatively small, nonsignificant reduction in *p,p'*-DDT, whereas *p,p'*-DDE, not present in the wettable powder, increased significantly from trace amounts at the onset to levels approaching 1 ppm. The *o,p'*-DDT component did not change significantly from the first sampling period and may be relatively less impervious to degradation by microorganisms than *p,p'*-DDT. Both *o,p'*-DDT and *p,p'*-DDT were highly persistent in soils, confirming the observations by Dimond et al. (1970).

The pathway for metabolism of *p,p'*-DDT in earthworms is more complicated because worms accumulate residues which have been partially subjected to degradation by soil microorganisms. There is considerable evidence that the microflora of the mammalian gastrointestinal tract can dechlorinate *p,p'*-DDT to *p,p'*-DDD (Braunberg and Beck 1968; McCulley et al. 1968). Whether or not earthworms contain similar microflora, they ingest soil which contains organisms capable of dechlorinating DDT to DDD under anaerobic conditions (Guenzi and Beard 1967, 1968). Based on the large quantities of *p,p'*-DDD in earthworms in our study, it appears that DDT is dechlorinated to DDD in earthworms. Presumably the DDD is further dechlorinated to DDE, although Davis (1971) was unable to detect this conversion. He also suggested that *p,p'*-DDT goes directly to *p,p'*-DDE in earthworms (Davis and French 1969; Davis 1971). The two hypotheses are not necessarily mutually exclusive and may reflect the different conditions of each study. The *o,p'*-DDT component in soils was not found in earthworms in the present study; *o,p'*-DDT has been reported in earthworms by others (Collett and Harrison 1968; Davis 1968; Wheatley and Hardman 1968; Davis and French 1969; Bailey et al. 1970; Gish 1970) but not in all samples. Davis (1971) has shown that both *o,p'*-DDT and *p,p'*-DDE appear in *A. caliginosa* after 15 days in *o,p'*-DDT treated soils.

Environmental levels of DDT resulting from a program to control Dutch elm disease were followed for about 15 months after the last application (Boykins 1964, 1966). We selected data from Boykins' study (1964) for time trend comparisons by using only the study areas that had soil and earthworm samples from every period. Our statistical analysis of his data showed that residues of DDD + DDT declined linearly with time ($P < 0.01$) in both soils and earthworms, whereas our soil residues did not change. In contrast to our study, seasonal fluctuations of DDD + DDT in earthworms were not evident, but such an effect would not have been obvious in only 15 months (see Table 3). Because Boykins (1964), using the Schecter-Haller analytical method (Schecter et al. 1945), quantitatively determined DDT at 600$\mu$, DDD and unknown amounts of other substances were included in the measurement but not DDE, which is read at 540$\mu$. This fact may partially account for the rapid decline in his estimates of total DDT. Dimond et al. (1970) found higher amounts of total DDT and metabolites in earthworms from forest soils receiving more frequent DDT application; however, no time trends were obvious. Ratios of residues in earthworms to residues in soils were less than 1.0, a situation that occurred only with *p,p'*-DDT in our study. In two studies, residues of DDT and metabolites in soils and earthworms were followed for 12 (Collett and Harrison 1968) and 15 (Bailey et al. 1970) months. Soil residues varied with the spraying program in the 15-month study (Bailey et al. 1970) but did not change in the 12-month study (Collett and Harrison 1968). Fluctuations in earthworm residues in both studies appeared to follow the spray program; no seasonal fluctuations could be determined from the sampling periods used in either study (Collett and Harrison 1968; Bailey et al. 1970).

### *Heptachlor Plots*

Two subsamples of wettable powder formulation with "25%" technical heptachlor averaged 28.5% active

ingredients including 23.0% heptachlor, 5.0% γ-chlordane, 0.4% α-chlordane, and less than 0.01% heptachlor epoxide. These compounds subsequently made up over 92% of the amount of all organochlorine insecticides in the soil from heptachlor-treated plots and 91% in the earthworms. The remainder was composed mostly of dieldrin, *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD.

Heptachlor and chlordane compounds remaining in soils and earthworms following the application of technical heptachlor are presented in Fig. 4. Each line represents the mean of the two replications (plots). Differences between replications were not significant for any compound in either soils or earthworms. Because α-chlordane was found infrequently and in small amounts, it is included only in combination with the other compounds in the total (Fig. 4D).

Residue differences among treatment levels for soils were significant for all individual compounds and the total. Differences among treatment levels for earthworms were significant only for heptachlor epoxide and total of heptachlor and chlordane compounds, and approached significance for γ-chlordane ($P = 0.06$) and heptachlor ($P = 0.12$). All compounds (and their total) in soils and earthworms increased linearly with increasing levels of application, except heptachlor in earthworms, which approached significance ($P = 0.06$).

Soil residue differences among sampling periods were significant for heptachlor, heptachlor epoxide, the combined total of all organochlorines ($P < 0.01$) and for γ-chlordane. Soil levels of heptachlor declined over the 2-year period, while those of heptachlor epoxide increased. Levels of γ-chlordane fluctuated during this time but did not decrease significantly. The total was significantly greater at the beginning before the breakdown of heptachlor and again at 2 years, apparently reflecting the increase in heptachlor epoxide (Table 2). The decline in heptachlor and the increase in heptachlor epoxide in soils were linear ($P < 0.01$) with time (Table 4).

In earthworms, quantities of all compounds and the combined total of all organochlorines differed ($P < 0.01$) among sampling periods (Table 3 and Fig. 4). Maximum heptachlor levels of 0.99, 1.7, and 3.2 ppm for 0.56, 2.24, and 8.97 kg ai/ha plots were recorded at 2 weeks. Levels declined until January, increased in March, and then disappeared. The January and March levels were significantly different from each other and both were less than the maximum at 2 weeks. Maximum residues of the remaining compounds, recorded in September (4 months after treatment), averaged 3.2, 11, and 30 ppm heptachlor epoxide; 1.4, 7.7, and 27 ppm γ-chlordane; and 4.6, 19, and 58 ppm total heplachlor and its metabolites from the respective 0.56, 2.24, and 8.97 kg ai/ha plots. Residues then declined from November to January, increased into May, declined through September to a new low in January, and increased again through March into May. The highs and lows were significantly different and, generally, less than the maximum recorded at 4 months. The decline of heptachlor at all treatment levels and of γ-chlordane at two levels was significantly linear ($P < 0.01$) with time (Table 4). Heptachlor epoxide and total residues in earthworms declined with time at the lower treatment levels but not at the 8.97 kg ai/ha level. Within individual sampling periods, total heptachlors and chlordanes in earthworms were significantly correlated with levels in soils, but not over the 2-year study, probably because of seasonal fluctuations of residues in earthworms. Ratios of residues in earthworms to residues in soils, as measured for dieldrin and DDT, were not different for replications or levels of application. Differences in ratios of residues in worms to residues in soils among the sampling periods were significant ($P < 0.01$) for heptachlor, heptachlor epoxide, γ-chlordane, and total residues. Levels of heptachlor in earthworms averaged 4.3 times those in soils at 2 weeks after application; thereafter the ratio gradually declined until, at 6 months, quantities in soil were greater than those in earthworms. Heptachlor epoxide in earthworms averaged 1,879 times the level in soil immediately after application, but by 2 weeks, differences were no longer significant. This initial difference probably was due to the more rapid conversion of heptachlor to its epoxide in worms than in soil. Maximum ratios of 75.6 for γ-chlordane and 38.0 for total residues were reached at 4 months and declined in a cyclic pattern similar to that exhibited by earthworm residues. Ratios were significantly cyclic for heptachlor, γ-chlordane, and total residues.

Total soil and earthworm residues were compared by stepwise regression with other variables measured in this study (Table 6). Percent organic matter and pH in soils appeared to be related to fluctuations of the total heptachlors and chlordanes in soils, but this relationship was not significant. Over 71.6% ($P < 0.01$) of the variability in the total heptachlors and chlordanes in earthworms was accounted for by the linear regression equation

$$\hat{Y} = 1.23695 + 0.67113\,X_1 - 0.03676\,X_2 + 0.08872\,X_3 + 0.27928\,X_4$$

where $\hat{Y}$ is the predicted logarithm of ppm of total residues in earthworms, $X_1$ is the logarithm of ppm in soils, $X_2$ is the percent soil moisture, $X_3$ is the total amount of rain falling during the 2 weeks before sampling, and $X_4$ is the percent lipid in earthworms. This equation describes only the existing relationship of total heptachlors and chlordanes in earthworms to other variables, but it may have predictive value.

Others have reported nonachlor (Lichtenstein et al. 1970), 1-hydroxychlordene (Bowman et al. 1965; Duffy and Wong 1967; Carter and Stringer 1970) and γ-chlordane (Harris et al. 1966; Duffy and Wong 1967; Saha and Stewart 1967; Saha et al. 1968; Gish 1970) in addition to heptachlor and heptachlor epoxide from heptachlor-treated soils. Heptachlor epoxide, chlordene, and 1-hydroxychlordene may result from microbial conversion of heptachlor (Miles et al. 1969), but only heptachlor, hepta-

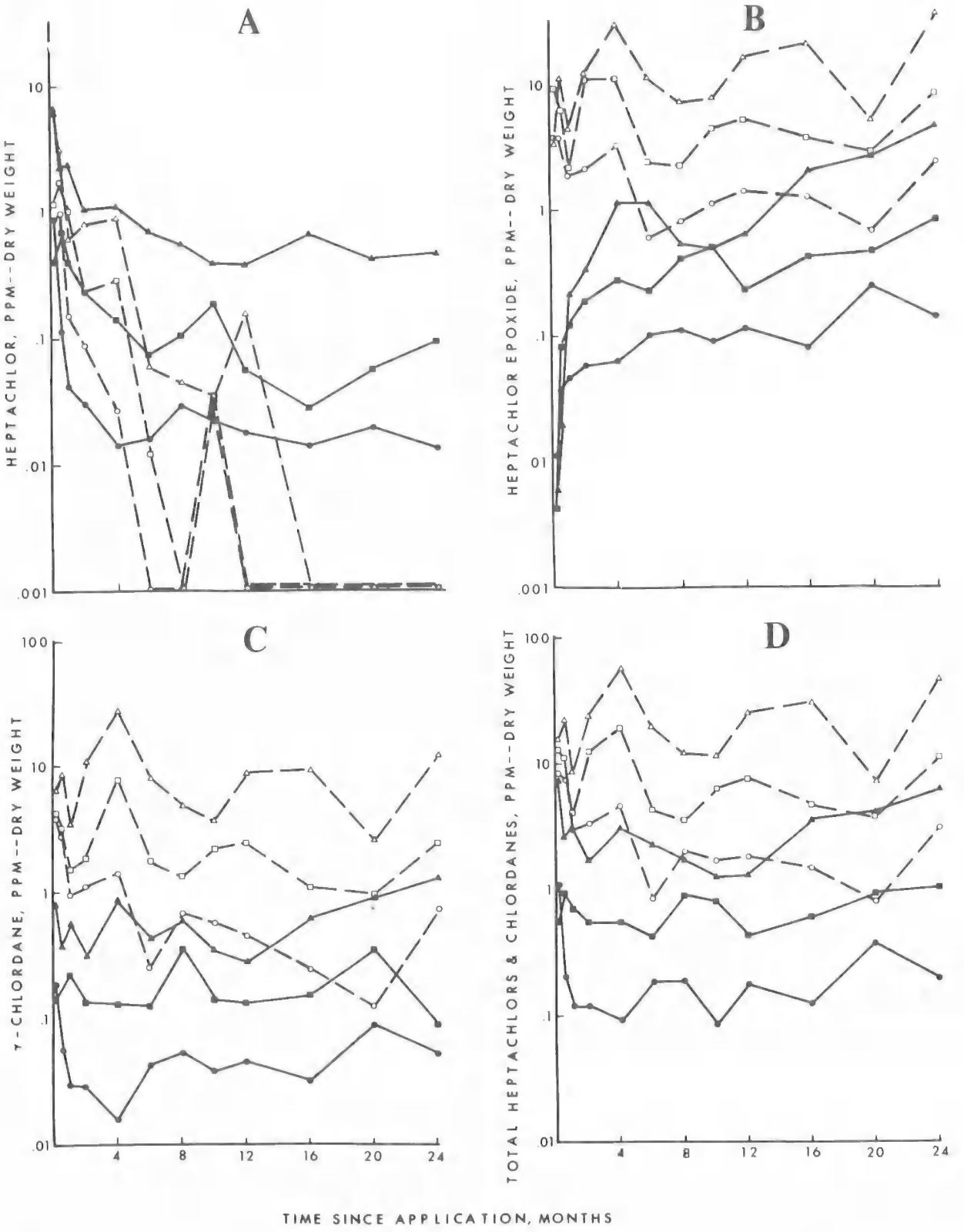


Fig. 4. Mean quantities of heptachlor (A), heptachlor epoxide (B), γ-chlordane (C), and heptachlor, heptachlor epoxide, α-chlordane, + γ-chlordane (D) in soils (solid lines) and earthworms (broken lines) at various times following a single application of 25% wettable powder heptachlor at 0.56 (circles), 2.24 (squares), or 8.97 (triangles) kg ai/ha.

chlor epoxide, α-chlordane, and γ-chlordane were found in soils in this study. Although small unidentified chromatographic peaks occurred infrequently, they were not 1-hydroxychlordene but may have been nonachlor or chlordene.

Earthworms appeared to metabolize heptachlor rapidly to its epoxide. The decline and disappearance of heptachlor residues in earthworms paralleled the decline of heptachlor in soils, the total process taking longer at higher rates of application. Earthworms contained no quantities of α-chlordane but seemed to accumulate γ-chlordane and store it without conversion. However, there are indications that on the DC-200 column (the main column used in this study) a metabolite of α- or γ-chlordane, oxychlordane, may appear as heptachlor epoxide (Polen et al. 1971). Oxychlordane was found in the fat of animals but not in soil or plants (Polen et al. 1971).

Heptachlor levels in earthworms declined in our study, whereas those of heptachlor epoxide increased. The total of heptachlor and heptachlor epoxide declined very little during the 2 years, confirming similar results of a study covering an 18-month period (Smith and Glasgow 1965). Heptachlor + heptachlor epoxide in earthworms from the 0.56 kg ai/ha plots was present in quantities similar to those reported by others for fields treated with similar amounts (Smith and Glasgow 1965; Stickel et al. 1965). Reports of γ-chlordane in earthworms were made by investigators using gas chromatographic methods (Gish 1970), but not from those using colorimetric methods (Smith and Glasgow 1965; Stickel et al. 1965).

### *Influence of Environmental Factors*

Determinations of moisture, organic content, and pH were made in all soil samples, as were moisture and lipids in earthworms (Table 6). These factors were compared with the total residues in respective soil and earthworm samples to study possible relationships. The associations, by chemicals, were discussed earlier.

Organic content was consistently correlated with soil residue levels for dieldrin, total DDT compounds, and total heptachlors and chlordanes ($r = 0.28$, $n = 72$), and to the amount of chemical applied. Levels of organic matter were greater at the 2.24-kg (4.1%) and 8.97-kg (4.1%) plots than at the 0.56 kg ai/ha plot (3.7%); perhaps this indicates that microorganisms that attack organic matter were inhibited by higher levels of applications.

The influence of organic content of soil on residue levels has been extensively reviewed (Bailey and White 1964; Wolcott 1970) and generally this factor has been more useful than others for predicting pesticide behavior in soils (Wolcott 1970). We observed with Wolcott (1970) that the addition of other factors to the multiple regression equation did very little to improve the predictive value.

Percent lipids were significantly lower in earthworms from plots which were mulched and rototilled (0.78%) than from those only mulched (0.90%). Moisture in earthworms from the 0.56 kg ai/ha plots (72.3%) averaged significantly less than that from the 2.24-kg plots (73.0%); and that from the 2.24-kg plots averaged significantly less than that from the 8.97-kg plots (73.7%). Moisture in earthworms from the DDT plots (72.5%) averaged significantly less than from the dieldrin (73.2%) or heptachlor plots (73.3%). Dieldrin, chlordane, and heptachlor have been demonstrated to be more poisonous to earthworms than DDT (Davey 1963; Van Der Drift 1963); heavier doses are more devastating than lighter ones (Davey 1963). Earthworms were noticeably more difficult to obtain in the heptachlor and dieldrin plots, especially those plots treated at 8.97 kg ai/ha, and it is possible that these insecticides may have interfered with water regulatory mechanisms.

All of the above factors fluctuated with time ($P < 0.01$) and were suspected to influence residue levels in earthworms. The influences of the individual factors on dieldrin, total DDT compounds, and total heptachlors and chlordanes were discussed under the individual chemicals. As would be expected, the amount of chemicals in the soils was of primary importance in determining quantities of residue in earthworms. The influence of the remaining factors varied slightly with the chemical.

A composite, predictive equation accounting for 80.0% of the variability in earthworm residues was based on the totals of all chemicals

$$\hat{Y} = -1.61711 + 0.66087\,X_1 - 0.02909\,X_2 + 0.03817\,X_3 + 0.07432\,X_4 + 0.35864\,X_5 - 0.01540\,X_6$$

where $\hat{Y}$ is the predicted logarithm of ppm total residues in earthworms, $X_1$ is the logarithm of ppm in soils, $X_2$ is months after treatment, $X_3$ is percent moisture in earthworm, $X_4$ is inches of rainfall during the 2 weeks before sampling, $X_5$ is percent lipids in earthworms, and $X_6$ is percent moisture in soil. The descriptive equation, like those for the totals of the individual test chemicals, may be useful for making general predictions of insecticide residues in earthworms at a specific time following application. The accuracy of such predictions has not been determined. The value of this equation may be limited by the methods used to estimate the other variables and may vary with chemicals other than the ones used in our study. If the other variables are to be determined by measurement, then it would be less complicated simply to measure the residues in earthworms.

### *Seasonal Fluctuations of Residues in Earthworms*

Residues in earthworms were higher, usually significantly so, at certain times of the year than at others. Those maximums generally were evident in late spring and occurred for all individual chemicals and the totals. The seasonal fluctuations in earthworm residues appear to coincide with seasonal behavioral phenomena of earthworms. Populations of *Allolobophora caliginosa* and *A. chlorotica* are almost wholly inactive during hot dry periods, partially active during January, and generally are closer to the surface in spring and fall than in middle to

late summer or in winter (Gerard 1967). *Lumbricus terrestris* appears to move faster in the evening during the summer months than in the winter (Bennett 1968). Not only do earthworms spend more time near the surface in the spring and fall, some species may spend several hours on the surface in the evenings; they also are more active during these times. This closer contact with the surface, the portion of the soil containing the greatest amount of insecticide residues, and their greater activity at this time may partially account for the larger residues in worms in the spring and fall.

Concentrations of some chemicals in the soil changed with time; *p,p'*-DDE and heptachlor epoxide increased whereas heptachlor decreased. Although dieldrin, *p,p'*-DDT, γ-chlordane, and total heptachlors and chlordanes fluctuated with time, there was no significant difference between quantities at the beginning and after 2 years. Without obvious decreases, neither chemical half-lives (Edwards 1966; Nash and Woolson 1967) nor 95% disappearance (Edwards 1966) could be estimated.

Heptachlor had disappeared from earthworms by 16 months. Residues of the other metabolites and their totals in earthworms generally were greatest from 2 to 4 months after application. After the maximum values were reached, residues declined, with a general increase in late spring and a decrease in the winter. These highs and lows coincided with reported general patterns of seasonal behavior of earthworms.

The 2.24-kg and 8.97-kg ai/ha rates depressed decomposition of soil organic matter, probably by affecting the organisms responsible for decomposition. Moisture retention of earthworms was enhanced by dieldrin and heptachlor and also increased with the concentration of insecticide in the soil. Observations on the scarcity of earthworms at the higher rates of application in the dieldrin and heptachlor plots suggest that insecticides may cause earthworm mortality. Maximum residues in earthworms did not occur immediately after chemical application, as one might expect, but about 2 months later for DDT and metabolites and 4 months later for dieldrin, heptachlor, and chlordane compounds. Thus, major effects of organochlorine insecticide applications on earthworm-eating vertebrates might not appear for several months after application.

Residue quantities in earthworms declined rapidly after the maximums, but secondary peaks generally occurred in late spring, when young birds would likely be fed earthworms. Thus, a potential hazard of a single application to earthworm-eating wildlife was still present 2 years after application. By not recognizing the seasonal variability of residues in earthworms, possible erroneous conclusions could be made that no acute poisoning hazard existed for earthworm-eating wildlife.

The trends in soil residues were few. With the exception of certain metabolites, the residues in soils were rather stable. Soil residues alone are rather poor indicators of the dynamics of the residues in earthworms, and undoubtedly in other soil fauna, that might be consumed by vertebrate species. The earthworm residues varied with a definite pattern coinciding with the periods of earthworm activity. Because earthworms use a larger area, both laterally and vertically, than would be represented by a single soil sample, they probably are better indicators of the insecticide residues in the terrestrial environment than are single or pooled soil samples.

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## References

Bailey, G. W., and J. L. White. 1964. Review of adsorption and desorption of organic pesticides by soil colloids, with implications concerning pesticide bioactivity. J. Agric. Food Chem. 12(4):324–332.

Bailey, S., P. J. Bunyan, D. M. Jennings, and A. Taylor. 1970. Hazards to wildlife from the use of DDT in orchards. Pestic. Sci. 1(2):66–69.

Baker, W. L. 1946. DDT and earthworm populations. J. Econ. Entomol. 39(3):404.

Barker, R. J. 1958. Notes on some ecological effects of DDT sprayed on elms. J. Wildl. Manage. 22(3):269–274.

Bennett, M. F. 1968. Persistent seasonal variations in the diurnal cycle of earthworms. Z. Vergleich. Physiol. 60(1):34–40.

Bowman, M. C., M. S. Schechter, and R. L. Carter. 1965. Insecticide behavior in soils, behavior of chlorinated insecticides in broad spectrum of soil types. J. Agric. Food Chem. 13(4): 360–365.

Boykins, E. A. 1964. DDT residues in the food chains of birds. Ph.D. Thesis. Michigan State University, East Lansing. 61 pp.

Boykins, E. A. 1966. DDT residues in the food chains of birds. Atlantic Nat. 21(1):18–25.

Braunberg, R. C., and V. Beck. 1968. Interaction of DDT and the gastrointestinal microflora of the rat. J. Agric. Food Chem. 16(3):451–453.

Caro, J. H., and A. W. Taylor. 1971. Pathways of loss of dieldrin from soils under field conditions. J. Agric. Food Chem. 19(2): 379–384.

Carter, F. L., and C. A. Stringer. 1970. Residues and degradation products of technical heptachlor in various soil types. J. Econ. Entomol. 63(2):625–628.

Cochran, W. G., and G. M. Cox. 1957. Experimental designs. Second edition. John Wiley & Sons, Inc., New York. 611 pp.

Collett, N., and D. L. Harrison. 1968. Some observations on the effects of using organochlorine sprays in an orchard. N.Z. J. Sci. 11(2):371–379.

Davey, S. P. 1963. Effects of chemicals on earthworms: a review of the literature. U.S. Fish Wildl. Serv., Spec. Sci. Rep.–Wildl. 74. 20 pp.

Davis, B. N. K. 1966. The uptake of organochlorine insecticides by invertebrates. Laboratory studies. Pages 55–56 *in* Monks Wood Experimental Station report for 1960–65.

Davis, B. N. K. 1968. The soil macrofauna and organochlorine insecticide residues in twelve agricultural sites near Huntingdon. Ann. Appl. Biol. 61(1):29–45.

Davis, B. N. K. 1971. Laboratory studies on the uptake of dieldrin and DDT by earthworms. Soil Biol. Biochem. 3(3):221–233.

Davis, B. N. K., and M. C. French. 1969. The accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields. Soil Biol. Biochem. 1(1):45–55.

Dimond, J. B., G. Y. Belyea, R. L. Kadunce, A. S. Getchell, and J. A. Blease. 1970. DDT residues in robins and earthworms associated with contaminated forest soils. Can. Entomol. 102(9):1122–1130.

Draper, N. R., and H. Smith. 1968. Applied regression analysis. John Wiley & Sons, Inc., New York. 407 pp.

Duffy, J. R., and N. Wong. 1967. Residues of organochlorine insecticides and their metabolites in soils in the Atlantic Provinces of Canada. J. Agric. Food Chem. 15(3):457–464.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11(1):1–42.

Edwards, C. A. 1965. Effects of pesticide residues on soil invertebrates and plants. Pages 239–261 *in* Ecology and industrial society: fifth symposium of the British Ecological Society. John Wiley & Sons, Inc., New York.

Edwards, C. A. 1966. Insecticide residues in soils. Residue Rev. 13:83–132.

Gerard, B. M. 1967. Factors affecting earthworms in pastures. J. Anim. Ecol. 36(1):235–252.

Gish, C. D. 1970. Organochlorine insecticide residues in soils and soil invertebrates from agricultural lands. Pestic. Monit. J. 3(4):241–252.

Guenzi, W. D., and W. E. Beard. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science 156(3778):1116–1117.

Guenzi, W. D., and W. E. Beard. 1968. Anaerobic conversion of DDT to DDD and aerobic stability of DDT in soil. Soil Sci. Soc. Am. Proc. 32(4): 522–524.

Harris, C. R., W. W. Sans, and J. R. W. Miles. 1966. Exploration studies on occurrence of organochlorine insecticide residues in agricultural soils in Southwestern Ontario. J. Agric. Food Chem. 14(4):398–403.

Hunt, L. B., and R. J. Sacho. 1969. Response of robins to DDT and methoxychlor. J. Wildl. Manage. 33(2):336–345.

Johnson, E. V. 1969. Robin-pesticide ecology on a commercial fruit farm. Ph.D. Thesis. Cornell University, Ithaca, N.Y. 96 pp.

Ko, W. H., and J. L. Lockwood. 1968. Conversion of DDT to DDD in soil and the effect of these compounds on soil microorganisms. Can. J. Microbiol. 14(10):1069–1073.

Korschgen, L. J. 1970. Soil-food-chain-pesticide wildlife relationships in aldrin-treated fields. J. Wildl. Manage. 34(1):186–199.

Korschgen, L. J. 1971. Degradation of aldrin after five annual applications. J. Wildl. Manage. 35(3):494–500.

Lichtenstein, E. P., K. R. Schultz, T. W. Fuhremann, and T. T. Liang. 1970. Degradation of aldrin and heptachlor in field soils during a ten-year period: translocation into crops. J. Agric. Food Chem. 18(1):100–106.

Luckmann, W. H., and G. C. Decker. 1960. A 5-year report of observations in the Japanese beetle control area at Sheldon, Illinois. J. Econ. Entomol. 53(5):821–827.

McCully, K. A., W. P. McKinley, and W. E. J. Phillips. 1968. The effect of the route of administration of single doses of *p,p'*-DDT on residues in adipose and liver tissues. J. Assoc. Off. Anal. Chem. 51(5):1050–1057.

Miles, J. R. W., C. M. Tu, and C. R. Harris. 1969. Metabolism of heptachlor and its degradation products by soil microorganisms. J. Econ. Entomol. 62(6):1334–1338.

Nash, R. G., and E. A. Woolson. 1967. Persistence of chlorinated hydrocarbon insecticides in soils. Science 157(3791):924–927.

Polen, P. B., M. Hester, and J. Benziger. 1971. Characterization of oxychlordane, animal metabolite of chlordane. Bull. Environ. Contam. Toxicol. 5(6):521–528.

Saha, J. G., C. H. Craig, and W. K. Janzen. 1968. Organochlorine insecticide residues in agricultural soil and legume crops in Northeastern Saskatchewan. J. Agric. Food Chem. 16(4):617–619.

Saha, J. G., and W. W. A. Stewart. 1967. Heptachlor, heptachlor epoxide, and gamma-chlordane residues in soil and rutabaga after soil and surface treatments with heptachlor. Can. J. Plant Sci. 47(1):79–88.

Schecter, M. S., S. B. Soloway, R. A. Hayes, and H. L. Haller. 1945. Colorimetric determination of DDT. Ind. Eng. Chem., Anal. Ed. 17(11):704–709.

Smith, R. D., and L. L. Glasgow. 1965. Effects of heptachlor on wildlife in Louisiana. Pages 140–154 *in* Proceedings of the 17th Annual Conference of the Southeastern Association of Game and Fish Commissioners.

Stickel, W. H., D. W. Hayne, and L. F. Stickel. 1965. Effects of heptachlor-contaminated earthworms on woodcocks. J. Wildl. Manage. 29(1):132–146.

Stringer, A., and J. A. Pickard. 1964. The DDT content of soil and earthworms in an apple orchard at Long Ashton. Pages 127–131 *in* Report of the Agriculture and Horticulture Research Station, University of Bristol for 1963.

Van Der Drift, J. 1963. De Invloed van Biociden op de Bodemfauna. [The influence of biocides on the soil fauna.] Neth. J. Plant Pathol. 69:188–199.

Wheatley, G. A., and J. A. Hardman. 1968. Organochlorine insecticide residues in earthworms from arable soils. J. Sci. Food Agric. 19(4):219–225.

Wolcott, A. R. 1970. Retention of pesticides by organic materials in soils. Pages 128–138 *in* Pesticides in the soil: Ecology, degradation and movement. Proceedings of Symposium, Michigan State University, February 25–27, 1970.

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